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(54) Title: **FLUOROGENIC ENZYME SUBSTRATES AND METHODS OF PREPARATION**

(57) Abstract: This invention relates to hydrolase fluorogenic substrates with improved cell permeability, methods for the preparation thereof, and methods of measuring activities of hydrolases, particularly in cell-based assays. The substrates easily diffuse into the cells, where they are enzymatically processed to yield photostable fluorescent products, and are particularly fitted for visualising enzyme-derived activities in cell-based assays.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, MEDLINE, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/04077 A (MOLECULAR PROBES INC) 4 March 1993 (1993-03-04) abstract; claims 1-65; figure 1	1-12
X	CHEMICAL ABSTRACTS, vol. 70, no. 17, 1969, Columbus, Ohio, US; abstract no.: 77907, B.M.BOLOTIN ET AL.: "2-Aryl-4H-3,1-benzoxazin-4-ones and their analogs. II. Some Reactions of O-Acylaminobenzamides." XP002257309 abstract & ZH. VSES. KHIM. OBSHCHEST., vol. 13, no. 4, 1968, pages 475-476, * registry number 22294-66-2 * ----- -/-	1-5
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *G* document member of the same patent family		
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Scott, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 2002, no. 05, 3 May 2002 (2002-05-03) & JP 2002 030085 A (ASAHI GLASS CO LTD;MITSUBISHI PHARMA CORP), 29 January 2002 (2002-01-29) abstract	1
Y	Z.DIWU ET AL.: "Spectral Properties and Biological Applications of ELF Enzyme Substrates that yield Fluorescent Precipitates at the Enzymatic Activity Sites." PROCEEDINGS OF SPIE - THE INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING 3602 ADVANCES IN FLUORESCENCE SENSING TECHNOLOGY IV, 1999, pages 265-274, XP008023235 * the whole document, but especially the abstract; table 2, compound 19 *	1-12
Y	WO 02/28841 A (MOLECULAR PROBES INC) 11 April 2002 (2002-04-11) the whole document	1-12
Y	ZHENJUN D ET AL: "Fluorescent Molecular Probes I. The Synthesis and Biological Properties of an ELF β beta-glucuronidase Substrate That Yields Fluorescent Precipitates at the Enzymatic Activity Sites" TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 53, no. 21, 26 May 1997 (1997-05-26), pages 7159-7164, XP004105695 ISSN: 0040-4020 the whole document	1-12
Y	J.J.NALEWAY ET AL.: "Synthesis and Use of New Fluorogenic Precipitating Substrates." TETRAHEDRON LETTERS., vol. 35, no. 46, 14 November 1994 (1994-11-14), pages 8569-8572, XP002230449 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4039 the whole document	1-12

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	M.ZHOU ET AL.: "A Fluorogenic Substrate for beta-Glucuronidase : Applications in Fluorometric, Polyacrylamide Gel and Histochemical Assays." JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, vol. 33, 1996, pages 197-205, XP002230450 the whole document	1-12
A	US 5 443 986 A (HAUGHLAND RICHARD P ET AL) 22 August 1995 (1995-08-22) cited in the application abstract	1-12
A	EP 0 722 937 A (BAYER AG) 24 July 1996 (1996-07-24) example 6	1
A	US 4 159 330 A (CORNO MARIA L ET AL) 26 June 1979 (1979-06-26) * example 6, 6-carboxy-2-(2'-ethoxy-5'-butyl-phenyl)-3,4-dihydro-4-oxo-quinazoline *	1
A	NL 6 409 642 A (AMERICAN CYANAMID COMPANY) 21 February 1966 (1966-02-21) * example 14 : 3-butyl-2-(2-hydroxy phenyl)-4(3)-quinazoline *	1

INTERNATIONAL SEARCH REPORT



Information on patent family members

International Application No

PCT/EP 03/51105

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9304077	A	04-03-1993	US 5316906 A AT 144524 T CA 2115461 C DE 69214841 D1 DE 69214841 T2 EP 0641351 A1 JP 8500961 T JP 3130316 B2 US 5443986 A WO 9304077 A1	31-05-1994 15-11-1996 03-12-1996 28-11-1996 28-05-1997 08-03-1995 06-02-1996 31-01-2001 22-08-1995 04-03-1993
JP 2002030085	A	29-01-2002	NONE	
WO 0228841	A	11-04-2002	AU 9650201 A CA 2424300 A1 EP 1322625 A2 JP 2004510767 T WO 0228841 A2 US 2002137068 A1	15-04-2002 11-04-2002 02-07-2003 08-04-2004 11-04-2002 26-09-2002
US 5443986	A	22-08-1995	US 5316906 A AT 144524 T CA 2115461 C DE 69214841 D1 DE 69214841 T2 EP 0641351 A1 JP 8500961 T JP 3130316 B2 WO 9304077 A1	31-05-1994 15-11-1996 03-12-1996 28-11-1996 28-05-1997 08-03-1995 06-02-1996 31-01-2001 04-03-1993
EP 0722937	A	24-07-1996	DE 19501481 A1 AU 704102 B2 AU 4098096 A BG 100293 A BR 9600148 A CA 2167345 A1 CN 1134417 A CZ 9600167 A3 EE 9600019 A EP 0722937 A1 FI 960227 A HR 960004 A1 HU 9600114 A2 IL 116770 A IN 184956 A1 JP 8253457 A MA 23780 A1 NO 960222 A NZ 280831 A PL 312352 A1 RO 117451 B1 RU 2158733 C2 SG 34377 A1 SK 7796 A3 TR 960705 A2 US 5721238 A ZA 9600397 A	25-07-1996 15-04-1999 25-07-1996 31-07-1996 06-01-1998 20-07-1996 30-10-1996 14-08-1996 15-08-1996 24-07-1996 20-07-1996 28-02-1998 30-12-1996 31-08-2000 07-10-2000 01-10-1996 01-10-1996 22-07-1996 28-10-1996 22-07-1996 29-03-2002 10-11-2000 06-12-1996 07-08-1996 21-08-1996 24-02-1998 28-08-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/51105

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4159330 A	26-06-1979	IT 1113561 B	20-01-1986
		IT 1118026 B	24-02-1986
		IT 1114419 B	27-01-1986
		AU 3134477 A	14-06-1979
		AU 512413 B2	09-10-1980
		BE 862201 A4	14-04-1978
		CA 1092118 A1	23-12-1980
		DE 2755716 A1	26-10-1978
		FR 2387967 A2	17-11-1978
		GB 1543874 A	11-04-1979
		GR 70041 A1	26-07-1982
		NL 7713862 A	23-10-1978
		NZ 185967 A	25-10-1979
		YU 300677 A1	31-08-1982
		ZA 7707410 A	25-10-1978
NL 6409642 A	21-02-1966	NONE	